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Characterization of *Erwinia amylovora* strains using random amplified polymorphic DNA fragments (RAPDs)

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M.T. MOMOL, E.A. MOMOL, W.F. LAMBOY, J.L. NORELLI, S.V. BEER AND H.S. ALDWINCKLE. 1997. The genetic diversity among 16 strains of *Erwinia amylovora*, chosen to represent different host plant origins and geographical regions, was investigated by RAPD analysis. One strain of *Erwinia herbicola* and one of *Agrobacterium vitis* were used as outgroups. Ninety-eight different RAPD fragments were produced by polymerase chain reaction amplification with six different 10-mer primers. RAPD banding profiles were found that enabled the *Erw. amylovora* strains to be distinguished from one another. Cluster analysis based on the number of RAPD fragments shared between strains showed that strains of *Erw. amylovora* isolated from subfamily Pomoideae formed a single group, whereas two strains from *Rubus* (subfamily Rosoideae) formed a second group. Two strains isolated from Asian pear on Hokkaido, Japan, formed a third group. Sets of RAPD fragments were identified that enabled each of the two host-range groups and one geographical region (Hokkaido) of *Erw. amylovora* strains to be unambiguously distinguished from one another and from the outgroups. This study shows that strains of *Erw. amylovora* exhibit genetic diversity detectable by RAPD analysis, and that molecular and statistical analysis of RAPD fragments can be used both to distinguish between strains and to determine relatedness between them.

INTRODUCTION

Fire blight caused by *Erwinia amylovora* (Burrill) Winslow *et al.* affects more than 130 species of plants in 39 genera of the plant family Rosaceae (van der Zwet and Keil 1979). It is a very serious disease of pome fruits and is present throughout North America, Europe and in numerous other countries of the world (van der Zwet and Beer 1995). Recently Beer *et al.* (1996) demonstrated that the 'bacterial shoot blight' pathogen from Hokkaido, Japan, is *Erw. amylovora*. In general, strains of *Erw. amylovora* are not host species specific. However, strains of *Erw. amylovora* that infect only *Rubus* species have been reported (Starr *et al.* 1951).

Several studies have indicated that strains of *Erw. amylovora* form a homogeneous group (Billing *et al.* 1961; Komagata *et al.* 1968; Paulin and Samson 1973). In the past, no characteristics have been found that can distinguish strains of different geographical origins, or strains that have been isolated either from different host plants or at different times (Vanneste 1995). Based on detailed taxonomic studies on the genus *Erwinia*, Dye (1968) found no major differences in biochemical characters or carbohydrate utilization among members of the *Erw. amylovora* group. Based on a serological study, Elrod (1941) concluded that *Erw. amylovora* was an exceedingly homogeneous species. Vantomme *et al.* (1982) tested 103 isolates of *Erw. amylovora* and found them to be quite homogeneous in their biochemical and protein electrophoretic characteristics, despite their different geographical and host origins. Except for strains from *Rubus* species, those having different geographical origins and isolated from different hosts were similar to each other with respect to percentage of fatty acid classes (van der Zwet and Wells 1993). *Rubus* strains showed a slight increase in cyclic acids compared to the other strains. Distinct RFLP profiles were obtained for *Rubus* strains that differed from those of Pomoideae strains when the *hrp* gene cluster was used as probe, and also two distinct groups of *Rubus* strains were detected (Laby and Beer 1992). Using rep-PCR and PCR ribotyping, McManus and Jones (1995) concluded that fruit tree strains of *Erw. amylovora* were genetically homogeneous and could be distinguished from strains isolated from *Rubus* species. In another study, five distinct groups of *Erw. amylovora* were identified based on carbon utilization as deter-

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mined with the BIOLOG™ system, hybridization of genomic DNA with the cloned *hrp* gene cluster of *Erw. amylovora* and PCR product from primers derived from pEA29 (Kim *et al.* 1995).

Assessment of genetic diversity in *Erw. amylovora* strains is important in epidemiology (short or long range pathogen dispersal), breeding for disease resistance, control of disease and plant quarantine. Data from genetic diversity studies could be used to monitor trends in the occurrence of pathogenic strains, to identify possible sources of infection, to assist in gene mapping, to aid in individual strain identification, to study population genetics of species, and to serve as characters in molecular phylogenetic studies (Bowditch *et al.* 1993). Thus, a technique that is able both to distinguish among *Erw. amylovora* strains and to provide information on relationships between them would be extremely valuable. Random amplified polymorphic DNA (RAPD) (Welsh *et al.* 1990; Williams *et al.* 1990) fragment analysis may be such a technique.

RAPD fingerprinting has been used as a sensitive and efficient method for distinguishing different strains of several other bacteria including *Escherichia coli* and *Helicobacter pylori* (Berg *et al.* 1994). For plant pathogenic prokaryotes, RAPD analysis has been used to study genetic relatedness among mycoplasma-like organisms associated with several geographically diverse grapevine yellow diseases (Chen *et al.* 1994), to determine phylogenetic relationships within *Xanthomonas campestris* (Smith *et al.* 1994), to distinguish strains of *Xanthomonas campestris* pv. *pelargonii* from 21 other *Xanthomonas* species and/or pathovars (Manulis *et al.* 1994), and to detect differences between California and eastern North American isolates of *Pseudomonas syringae* pv. *apii* (Little *et al.* 1994). RAPD analysis was used to reveal genetic and phenotypic variation of *Agrobacterium* biovars (Irelan 1994). Verification of strain identity of *Agrobacterium vitis* was achieved by RAPD analysis of total genomic DNA (Burr *et al.* 1995). RAPD analysis also has provided markers to differentiate races of several plant pathogenic fungi (Assigbetse *et al.* 1994).

The objective of our research was to use RAPDs to unambiguously identify ('fingerprint') and determine the relatedness of 16 different strains of *Erw. amylovora*, as well as to determine if RAPD data support the grouping of strains based upon the host plants from which they were isolated. Preliminary aspects of this study have been published (Momol *et al.* 1995, 1996).

MATERIALS AND METHODS

Bacterial strains

The strains of *Erw. amylovora* used in this study originated from various regions in North America, Europe and Hokkaido, Japan, and were isolated from cultivars of apple (*Malus* × *domestica* Borkh.), European pear (*Pyrus communis* L.), Asian pear (*Pyrus pyrifolia* (Burm.f.) Nak., cultivar 'Mishirazu') and *Rubus* spp. (raspberry) (Table 1). *Agrobacterium vitis* was isolated from grape. Identification of all strains of *Erw. amylovora* was confirmed by PCR amplification of a 0·9-kb fragment of pEA29 (Bereswill *et al.* 1992). All strains were cultured routinely at 28°C on nutrient agar (NA). Liquid cultures for DNA extraction were grown in Luria–Bertani medium (Sambrook *et al.* 1989) at 28°C. Bacterial strains were stored at −80°C in 50% glycerol + 50% Nutrient Yeast Glucose Broth.

RAPD assays

RAPD procedure was optimized for primer concentration, template concentration and annealing temperature; all were tested to reproduce consistent fragments. Results showed that the procedure described below, using annealing temperature of 42°C, gave the most consistent fragments.

Bacterial DNA was extracted as described by Rudner *et al.* (1994). DNA was quantified using a GeneQuant (Pharmacia) DNA calculator. For RAPD analysis, PCR (Innis *et al.* 1990) amplifications were carried out in 100-μl volumes and contained 50 ng of genomic DNA, 2·5 mmol l^{-1} $MgCl_2$, 50 pmol of primer, 2·5 U of *Taq* DNA polymerase (Promega), 0·1 mmol l^{-1} of each deoxynucleoside triphosphate (dNTP) (Boehringer) in 10 mmol l^{-1} Tris–HCl (pH 9·0), and 50 mmol l^{-1} KCl, under three drops of mineral oil. Sterile water was used as DNA-negative control in every PCR run. Amplification was performed in a thermal cycler (PTC-100, MJ Research, Watertown, MA) programmed for one cycle of 2 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 42°C and 2 min at 72°C and final extension for 5 min at 72°C. Twenty-four 10-mer arbitrary primers (obtained from Genosys, The Woodlands, TX, and Operon Technologies, Alameda, CA) were tested, and RAPD fragments produced by six of the primers were chosen for RAPD analysis (Table 2). Amplification reactions were conducted with each primer on the DNA of 16 strains of *Erw. amylovora*, and one strain each of *Erw. herbicola* and *Ag. vitis*. Two independent amplification reactions were carried out for each strain. Ten μl of the amplification products were resolved by electrophoresis on 1·5% agarose (0·5% NuSieve GTC agarose, FMC; 1% Ultra Pure agarose, Gibco BRL) gels and were stained with ethidium bromide and photographed in u.v. light. Presence and absence of the 98 different RAPD bands produced from the six primers were scored visually from the resulting photographs.

Cluster and fingerprinting analysis

Using the computer program SIMFIN (SIMilarity/FINgerprinting), written by one of the authors (WFL) in the

Table 1 *Erwinia amylovora* strains and outgroup bacteria used in this study

Strain		Host	Location	Isolator
Pomoideae				
1*	E4001A	apple (R.I. Greening)	Canada	W.G. Bonn
2	E2002A	apple (Jonathan)	Canada	W.G. Bonn
3	Ea273	apple (R.I. Greening)	New York	S.V. Beer
4	Ea225	apple (20 Oz.)	New York	S.V. Beer
5	WE07	pear	California	S.V. Thomson
6	Ea 1/79	apple (James Grieve)	Germany	W. Zeller
7	P42	pear	England	J.E. Crosse
8	88-100	pear	Washington	R. Roberts
9	EaOR1	pear	Oregon	V. Stockwell
10	122wt	apple (Jonathan)	Michigan	D. Ritchie
11	CFBP 2045	apple (R. des Reinettes)	France	J.P. Paulin
Rubus				
12	Ea528†	raspberry	Maine	D. Folsom
13	Ea416†	raspberry‡	Unknown	Unknown
14	Ea510†	raspberry	Canada	I.R. Evans
Hokkaido				
15	7971 (1)	Asian pear	Hokkaido, Japan	A. Tanii
16	TP9405	Asian pear	Hokkaido, Japan	A. Tanii
Outgroup				
17	*Erwinia herbicola* (Eh112YN)			S.V. Beer
18	*Agrobacterium vitis* (975)			T.J. Burr

* Same number has been used in other Tables and Figures for strains.
† This number is given by S.V. Beer and it is not the original designation. Ea528, ICMP 1539; Ea510, BR89-FR41.
‡ Pathogenicity to *Rubus* confirmed, not pathogenic on Pomoideae.

GAUSS programming language (Edlefsen and Jones 1986), genetic similarities between strains and groups of strains were computed from presence/absence data for RAPD fragments by the method of Nei and Li (1979). The Nei–Li similarity coefficient is preferable to either the Jaccard coefficient or the simple matching coefficient (Sneath and Sokal 1973) for use with RAPD data, because it is least affected by RAPD artefacts (Lamboy 1994a,b). For a pair of strains, the Nei–Li coefficient is the number of fragments that are present in both strains divided by the average number of fragments present in the two strains. To determine relationships between strains or groups of strains, these similarities were used in the unweighted pair group method using arithmetic means (UPGMA) cluster analysis procedure (Sneath and Sokal 1973), an option of the computer program NTSYS-pc (Rohlf 1988).

Table 2 RAPD code and sequence of bands produced by the six primers used in random amplified polymorphic DNA (RAPD) analysis of *Erwinia amylovora*

RAPD code	Sequence (5′–3′)	Bands produced*
CUGEA-1	TCGCCAGCGA	1–16
CUGEA-2	GTTGCGATCC	17–38
CUGEA-3	GCGGTACCCG	39–56
CUGEA-4	GCGAATTCCG	57–65
CUGEA-5	CGATCGATGC	66–86
CUGEA-6	GGAAGCTTCG	87–98

* Numbers denote unique bands of different (random) size.

The determination of RAPD fingerprints also was carried out using the SIMFIN program. For two strains to be distinguishable from one another, there must exist one or more RAPD fragments that are always present in one of the strains and always absent in the other. In order for two groups of strains to be distinguishable from one another, there must exist one or more RAPD fragments that are present in all of the strains belonging to one of the groups and are absent from all of the strains belonging to the other group. SIMFIN determines whether any two pairs of strains or groups of strains can be distinguished by means of their RAPD frag-

ments. A more detailed description of the procedure can be found in Ren *et al.* (1995).

Three statistical hypothesis tests were conducted to test whether any two of the three *a priori* groups of *Erw. amylovora* might belong to the same group based on their between-group Nei–Li similarities. Assuming random sampling of strains and assuming that the computed similarity values followed an approximately binomial distribution that, because of the large sample size (98 fragments), can be adequately approximated by a normal distribution, the hypothesis tests were conducted by comparing the between-group similarity of two groups against the weighted average of the within-group similarities of the two groups. If the average of the between-groups similarity is not statistically significantly different from the weighted average of the within-groups similarities for the same two groups, then it can be concluded that the groups are not significantly different from one another, and separation of strains into two groups is unwarranted (Table 5).

RESULTS

Identification of all *Erw. amylovora* strains used in this study were confirmed by PCR (Bereswill *et al.* 1992). Product sizes larger than 0·9 kb were detected for some *Rubus* and Hokkaido strains as has been reported by others (Kim *et al.* 1996).

PCR amplification using the six selected primers resulted in the production of 98 different RAPD fragments. Two independent amplifications of products resulted in the same fragment patterns. Representative sets of amplification products with six primers are shown in the gel photographs (Fig. 1). RAPD profiles (sets of RAPD bands) produced from genomic DNA unambiguously distinguished all 16 strains of *Erw. amylovora* from each other (Table 3). For example, for two of the most closely related strains, E4001A and E2002A, band number 71, generated by primer CUGEA-5, distinguished them; it was present in strain E4001A and absent from strain E2002A. The two most distantly related strains of *Erw. amylovora*, Ea510 and 7971(1), differed by 50 bands.

Sets of RAPD fragments were detected that distinguished all three *a priori* groups of *Erw. amylovora* strains from one another (Table 4). For example, bands 60 and 79 were present in all members of the Pomoideae group and were absent for all members of the *Rubus* group, while band 21 was present in all members of the *Rubus* group and absent in all members of the Pomoideae group.

The number of RAPD fragments for which a pair of strains differ (one strain possesses the fragment and the other lacks it) is shown in the upper triangle of the matrix in Table 3. Even without a mathematical or statistical analysis, these results suggest that Pomoideae strains Ea4001A, Ea2002A, Ea273, Ea225, WE07, P42, 88–100, EaOR1, 122wt and CFBP 2045, are closely related. The Pomoideae strain Ea1/79 is more distantly related to the others in the group, but it is still more similar to the Pomoideae group than it is to any of the other strains.

Of the *Rubus* strains, Ea416 shares the greatest number of bands with the Pomoideae group, and Ea528 shares an intermediate number. Strain Ea510 shares the fewest, suggesting that it is the *Rubus* strain in the study that is most distantly related to the Pomoideae group.

The Hokkaido strains, 7971(1) and TP9405, share approximately the same number of bands with the members of the Pomoideae and the *Rubus* groups, and *Erw. herbicola*. Overall, *Ag. vitis* shares the fewest number of bands with the other strains, as would be expected from its taxonomic placement in a separate genus and family.

The Nei–Li similarities between all 16 strains and two outgroups are displayed in the lower triangle in Table 3. Two phenograms were produced from these data by UPGMA cluster analysis. Only one is shown (Fig. 2), since the phenograms are identical except for the relationships between strains E4001A, E2002A and Ea273. In Fig. 2, strains E4001A and E2002A form a cluster to which Ea273 is then added. In the phenogram not shown, strains E2002A and Ea273 first form a cluster, and E4001A is added later. Apart from these differences the phenograms are identical. The cluster analysis confirms what was suggested by the data on number of band differences (Table 3); namely, that strains E4001A, E2002A, Ea273, Ea225, WE07, P42, 88–100, EaOR1, 122wt and CFBP 2045 form a closely related group; all pairs of strains within this group have similiarities, $s > 0{\cdot}900$. The remaining Pomoideae strain, Ea 1/79, also is most closely related to that group, but joins it at a lower level of similarity ($s = 0{\cdot}830$).

Two of the *Rubus* strains (Ea510 and Ea528) are mutually more closely related to one another (Fig. 1), than they are to any other strain. Based on similarities, Ea416 is more similar to the Pomoideae group ($s = 0{\cdot}791$) than it is to the other *Rubus* strains (Ea510 and Ea528). A similar result was reported earlier (Laby and Beer 1992; Kim *et al.* 1995). These independent results based on different tests suggest that the *Rubus* strains might belong to more than one subgroup.

The two strains isolated from Hokkaido are most closely related to one another ($s = 0{\cdot}613$), but are approximately as distantly related from one another as the two most dissimilar strains in the Pomoideae-*Rubus* group (strains Ea1/79 and Ea510 have $s = 0{\cdot}551$). Thus, the Hokkaido strains may represent a distinct, but diverse group among the 16 strains of *Erw. amylovora* that were examined.

After the 18 strains were classified into five *a priori* groups, the Pomoideae, the *Rubus*, the Hokkaido (Asian pears), *Erw. herbicola* and *Ag. vitis*, bands were found that distinguished all groups from one another (Table 4, upper triangle). The Pomoideae and the *Rubus* groups are most similar, judging by number of RAPD fragments for which they differ. The cluster analysis (Fig. 3) of the five groups shows clearly that

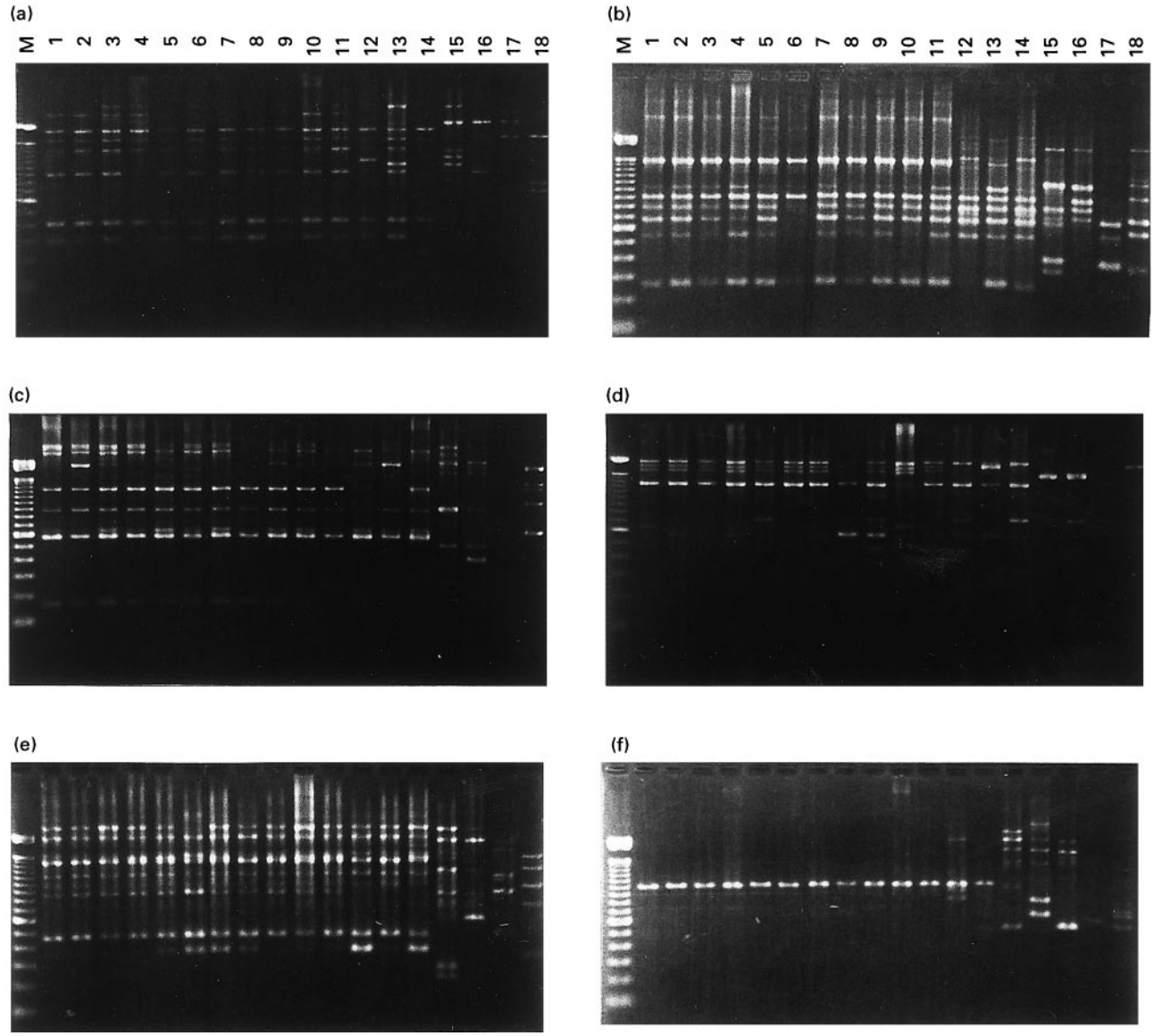

Fig. 1 Gels stained with ethidium bromide showing PCR amplification products generated from the *Erwinia amylovora* strains and outgroup bacteria with primers: (a) CUGEA-1, (b) CUGEA-2, (c) CUGEA-3, (d) CUGEA-4, (e) CUGEA-5 and (f) CUGEA-6. Strain designations: 1, E4001A; 2, E2002A; 3, Ea273; 4, Ea225; 5, WE07; 6, Ea1/79; 7, P42; 8, 88–100; 9, EaOR1; 10, 122wt; 11, CFBP 2045; 12, Ea528; 13, Ea416; 14, Ea510; 15, 7971(1); 16, TP9405; 17, *Erw. herbicola*; 18, *Agrobacterium vitis*. M = 100 bp DNA marker

the Pomoideae and the *Rubus* groups are much more closely related to each other than they are to the other groups or than the other groups are related to one another. The two Hokkaido strains are most similar first to one another, and then to (in order of decreasing similarity), the *Rubus* group, the Pomoideae, *Erw. herbicola* and *Ag. vitis*.

The results of statistical hypothesis tests to determine if any of the three *a priori Erw. amylovora* groups could be combined are shown in Table 5. All of the hypothesis tests were significant at a *P*-level $<0{\cdot}00001$, indicating that all three *a priori* groups were highly significantly different from one another.

DISCUSSION

We observed genetic diversity within a collection of 16 *Erw. amylovora* strains from USA, Europe and Japan, based on RAPD analysis. Strains were classified into three RAPD groups: Pomoideae, *Rubus* and Hokkaido (Japan). *Rubus* and Hokkaido groups each formed two subgroups. Therefore

Table 3 Nei–Li similarities between strains in the lower triangle, and the number of RAPD fragments for which the strains differ in the upper triangle

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1·000	1	2	5	6	10	5	6	4	3	6	17	12	28	48	46	42	52
2	0·986	1·000	1	4	5	11	4	5	3	2	5	18	13	29	47	45	41	53
3	0·971	0·986	1·000	3	6	10	5	6	4	3	4	17	12	30	46	46	42	54
4	0·928	0·941	0·957	1·000	5	11	4	7	5	4	3	20	15	27	47	49	41	53
5	0·914	0·928	0·914	0·928	1·000	12	1	4	2	5	6	17	16	24	50	46	42	54
6	0·853	0·836	0·853	0·836	0·824	1·000	11	12	14	11	12	19	18	30	50	48	44	50
7	0·928	0·941	0·928	0·941	0·986	0·836	1·000	3	3	4	5	18	15	25	49	47	41	53
8	0·909	0·923	0·909	0·892	0·939	0·813	0·954	1·000	6	7	6	17	14	26	50	44	38	50
9	0·943	0·957	0·943	0·928	0·971	0·794	0·957	0·909	1·000	3	6	17	14	26	48	44	42	54
10	0·957	0·971	0·957	0·941	0·928	0·836	0·941	0·892	0·957	1·000	5	20	15	29	47	45	43	53
11	0·912	0·925	0·941	0·955	0·912	0·818	0·925	0·906	0·912	0·925	1·000	19	14	28	48	46	40	54
12	0·761	0·743	0·761	0·714	0·761	0·725	0·743	0·746	0·761	0·714	0·725	1·000	15	17	45	43	43	51
13	0·829	0·812	0·829	0·783	0·771	0·735	0·783	0·788	0·800	0·783	0·794	0·789	1·000	26	46	44	38	52
14	0·611	0·592	0·583	0·620	0·667	0·571	0·648	0·618	0·639	0·592	0·600	0·767	0·639	1·000	50	42	42	50
15	0·314	0·319	0·343	0·319	0·286	0·265	0·290	0·242	0·314	0·319	0·294	0·366	0·343	0·306	1·000	24	40	48
16	0·258	0·262	0·258	0·197	0·258	0·200	0·230	0·241	0·290	0·262	0·233	0·317	0·290	0·344	0·613	1·000	36	44
17	0·160	0·163	0·160	0·163	0·160	0·083	0·163	0·174	0·160	0·122	0·167	0·157	0·240	0·192	0·200	0·143	1·000	36
18	0·103	0·070	0·069	0·070	0·069	0·107	0·070	0·074	0·069	0·070	0·036	0·136	0·103	0·167	0·172	0·120	0·053	1·000

Strain designations: 1, E4001A; 2, E2002A; 3, Ea273; 4, Ea225; 5, WE07; 6, Ea1/79; 7, P42; 8, 88–100; 9, EaOR1; 10, 122wt; 11, CFBP 2045; 12, Ea528; 13, Ea416; 14, Ea510; 15, 7971(1); 16, TP9405; 17, *Erwinia herbicola*; 18, *Agrobacterium vitis*.

Table 4 Matrix showing the Nei–Li similarities between groups of strains in the lower triangle, and the number of RAPD fragments for which the groups of strains differ in the upper triangle (the within-group similarities are shown on the main diagonal of the matrix in bold type)

	Pomoideae	*Rubus*	Hokkaido	*Erwinia herbicola*	*Agrobacterium vitis*
Pomoideae	**0·917**	3	29	32	42
Rubus	0·715	**0·732**	22	27	37
Hokkaido	0·272	0·328	**0·613**	26	34
Erw. herbicola	0·152	0·196	0·171	**1·000**	36
Ag. vitis	0·073	0·135	0·146	0·053	**1·000**

all strains could be classified into five distinct groups and subgroups: Pomoideae (all apple and pear strains), *Rubus* I (Ea416), *Rubus* II (Ea528 and Ea510), Hokkaido I (7971(1)) and Hokkaido II (TP9405). The grouping of strains by RAPD is consistent with the finding of Kim *et al.* (1996) who characterized *Erw. amylovora* strains based on carbon utilization as determined with the BIOLOG™ system, hybridization of genomic DNA with cloned *hrp* gene cluster of *Erw. amylovora*, and PCR product from primers derived from pEA29. The data presented here indicate that, for *Erw. amylovora*,

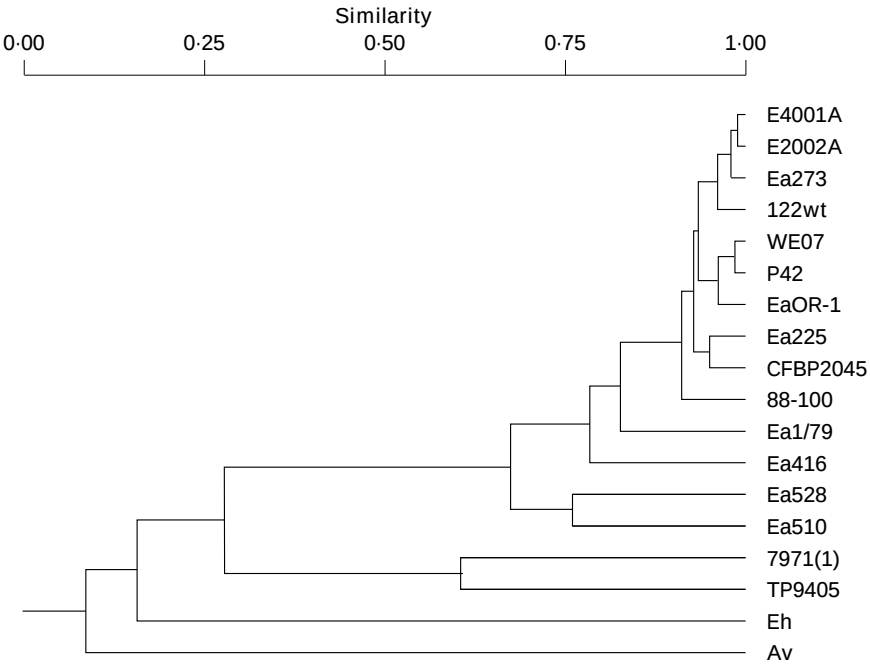

Fig. 2 Phenogram from UPGMA clustering of strains based on values of Nei–Li similarity coefficients. Strain designations: 1, E4001A; 2, E2002A; 3, Ea273; 4, Ea225; 5, WE07; 6, Ea1/79; 7, P42; 8, 88–100; 9, EaOR1; 10, 122wt; 11, CFBP 2045; 12, Ea528; 13, Ea416; 14, Ea510; 15, 7971(1); 16, TP9405; 17, *Erwinia herbicola* (Eh); 18, *Agrobacterium vitis* (Av)

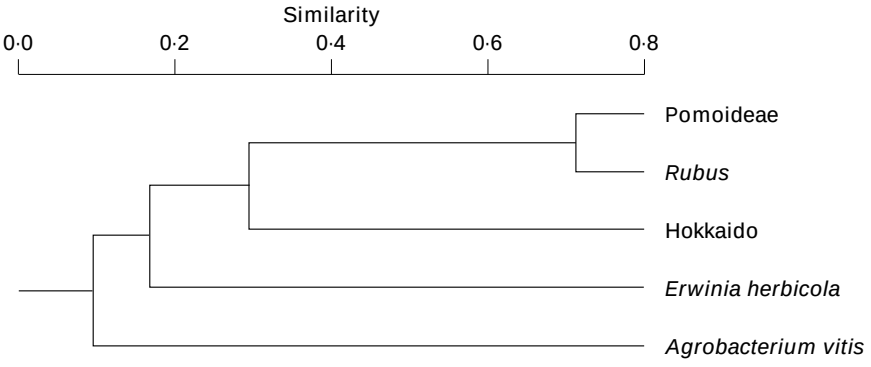

Fig. 3 Phenogram from UPGMA clustering of *a priori* groups of strains based on values of Nei–Li similarity coefficients

Table 5 Results of statistical tests* to determine if any of the three *a priori* groups of *Erwinia amylovora* might be combined

Groups	*z*-Statistic	Significance level
Pomoideae–*Rubus*	50·5	< 0·00001
Pomoideae–Hokkaido	68·9	< 0·00001
Rubus–Hokkaido	16·3	< 0·00001

* Test determines if the level of between-group similarity of the two groups is statistically equivalent to the weighted average of the within-group similarities of the two groups.

RAPDs have clear advantages over *hrp* gene RFLP (Laby and Beer 1992), rep-PCR and PCR ribotyping (McManus and Jones 1995) for generation of data suitable for analysis of the similarities and differences between strains. RFLP analysis and PCR ribotyping produced too few bands to provide sufficient data to distinguish different strains. Although rep-PCR produced several bands there was less difference in the banding pattern between different strains.

We have confirmed and extended previous studies that have shown differences between strains of *Erw. amylovora* isolated from *Rubus* and all other hosts. In one earlier study, three *Erw. amylovora* strains LMG 2083, 2084 and 2085 isolated from *Rubus*, showed a negative green pear test that distinguished them from most pathogenic *Erw. amylovora* strains (Vantomme *et al.* 1986). Molecular genetic analyses of strains of *Erw. amylovora* isolated from and infecting only *Rubus* species and those isolated from a variety of Pomoideae plants by Laby and Beer (1992), revealed differences in *hrp* genes of the strains. Additional studies have shown that *Rubus* strains were different from Pomoideae strains based on their host specificity (Starr *et al.* 1951), hypersensitive reaction (Vantomme *et al.* 1986), fatty acid class analysis (van der Zwet and Wells 1993) and rep-PCR and PCR ribotyping (McManus and Jones 1995). The RAPD results from this study also indicate that *Rubus* strains form a distinct group, which form two subgroups. Two distinct subgroups of *Erw. amylovora* strains isolated from *Rubus* spp. were also observed in other studies (Laby and Beer 1992; Kim *et al.* 1995; McManus and Jones 1995).

Previous studies indicated that strains of *Erw. amylovora* isolated from Asian pear in Japan differed significantly from strains infecting other host plants. A disease named bacterial shoot blight of pear (BSBP) was reported on twigs of the Asian pear cultivar 'Mishirazu', causing blight of blossoms, young fruits, leaves and shoots (Goto 1992). The symptoms of this disease were identical to those of fire blight, and the pathogen was reported to be similar to *Erw. amylovora* except for some bacteriological and serological properties (Goto 1992). Although this bacterium infected certain Asian pears, it was not reported to infect cultivars of apple, suggesting that there are some significant biological differences between it and other strains of *Erw. amylovora* and Goto stated 'it is considered to be a distinct pathovar of *Erw. amylovora*'. Recent results indicate that BSBP is fire blight caused by strains of *Erw. amylovora* that differ by several criteria from strains isolated from Pomoideae in other countries (Beer *et al.* 1996).

RAPD analysis of the strains isolated from Asian pear on Hokkaido, Japan, used in this study also indicate that they form a distinct third group of *Erw. amylovora* strains. Judging by the number of bands for which the groups differ, the Hokkaido strains are approximately equally dissimilar from the Pomoideae and *Rubus* groups, and *Erw. herbicola* (28, 21 and 26 bands, respectively). Surprisingly, the Hokkaido strains themselves differ from each other by 24 fragments, indicating that there may be two subgroups within the Hokkaido strains (Table 3). Kim *et al.* (1995) also found two subgroups within Hokkaido strains.

Other studies have also shown differences among Pomoideae strains. For example, the differential virulence of strain E4001A relative to several other strains tested for the apple cultivar Quinte was demonstrated (Norelli *et al.* 1984). In addition, some strains of *Erw. amylovora* are resistant to streptomycin (Miller and Schroth 1972; Chiou and Jones 1991).

The successful use of RAPD fragments in the identification and determination of relatedness between strains of *Erw. amylovora* suggests other potential uses for this technique. Specific polymorphic RAPD fragments could be used to generate strain-specific or RAPD group-specific probes. Such probes might identify bacterial strains that can cause fire blight on specific host plants. In ecological studies of *Erw. amylovora* on Pomoideae blossoms, RAPD fingerprinting could be used to study site competition between different strains of *Erw. amylovora* compared to a biological control agent. RAPDs should be useful in the analysis of the level of similarity among strains that originated in different geographical areas and on different hosts. This would allow an objective evaluation of the risk of non-indigenous strains of *Erw. amylovora* to new apple or pear growing areas to be made. Because RAPDs can be used for differentiating strains of *Erw. amylovora* that infect different hosts, they could be used for monitoring the pathogenic capability of strains of *Erw. amylovora* that may pose threats to the fruit industry in any given region.

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